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Oral Acute and Subacute Toxicity Studies of Decursin and Decursinol Angelate of *Angelica gigas* Nakai

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Accepted 22 April 2009

Abstract

In this study, we assessed the acute and subacute toxicity of *Angelica gigas* Nakai (*A. gigas* Nakai) extracts, which are comprised of decursin and decursinol angelate (D/DA) in rats. For the oral acute toxicity test, Sprague-Dawley (SD) male and female rats were gavaged with two doses of D/DA (200 and 2,000 mg/kg body weight) and then observed for any toxic symptoms for 2 weeks. The LD_{50} value for the rats was greater than 2,000 mg/kg body weight for both male and female rats, which indicates that there were no toxic symptoms induced by doses of up to 2,000 mg/kg body weight. For the subacute toxicity study, rats were treated with D/DA at doses of 2 and 20 mg/kg body weight once a day for 30 days. There were no significant changes in body weight and food intake observed during the subacute toxicity study. In addition, no differences were observed between the control and treated groups when urinalysis was conducted or when hematology and biochemical parameters were evaluated. Finally, histopathological examination of the organs did not reveal any lesions in the control or treated groups. Taken together, these findings indicate that D/DA is safe and non-toxic.

Keywords: Acute and Subacute toxicity, *Angelica gigas* Nakai, Decursin, Decursinol angelate

A. gigas Nakai (Cham-Dang-Gui in Korean) is a Korean traditional herbal medicine that is one of the most popular herbal medicines used in Asian countries, including Korea, Japan and China. *A. gigas* Nakai is also marketed as a functional food product in Europe and America. *A. gigas* Nakai of Korea is quite distinct in that it has deep purple flowers while Japan and China Angelica have white flowers[1]. *A. gigas* Nakai has been studied extensively and found to contain a variety of substances including coumarins[2,3]. Coumarins are comprised of D/DA, which has been used as a traditional medicine for the treatment of anemia, as a sedative, and as an anodyne or a tonic agent[4]. In addition, *A. gigas* Nakai has been widely used for the treatment of dysmenorrhea, amenorrhea, menopausal syndromes, abdominal pain, injuries, migraine headaches and arthritis. Furthermore, *A. gigas* Nakai is known to exert antibacterial and antiamnestic effects as well to induce inhibitory effects against acetylcholinesterase, depression of cardiac contraction, and activation of protein kinase C[5-9]. There are many herbal medicines that are used in functional food products. The subjects of great concern at present pose a genetic hazard in herb medicine. Recently, methods for improving the storage conditions and improving the quality of herbal medicines for supply and circulation have been evaluated. All drugs are toxic at higher doses; therefore, it is important to access their safety and efficacy prior to use. Accordingly, toxicological experiments are generally conducted in animals to develop and establish the safety and efficacy of new drugs. The data generated from such experiments is then utilized to determine if a new drug is suitable for clinical use or not[10]. Previously, we reported the genotoxicity of D/DA[11]. However, to date, there has been no information regarding acute and subacute toxicity of D/DA. Therefore, we conducted this acute and subacute study of D/DA in SD rats to determine its effects on morphology, gross behavior, and body weight, as well as to identify any histopathological, biochemical or hematological changes.

Acute Toxicity Study

In the acute toxicity test doses of 200 or 2,000 mg/kg body weight of D/DA, the rats did not show any

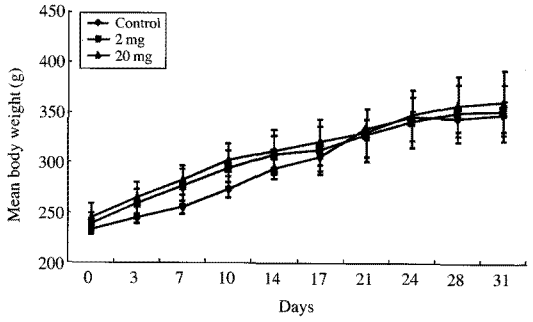

Figure 1. Changes in the body weights of male rats following oral administration of D/DA for 30 days. Results are the means ± S.D. (n=5).

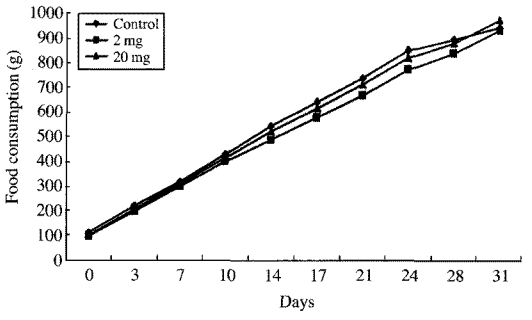

Figure 3. The cumulative food consumption of male rats administered D/DA orally for 30 days. Results are the means ± S.D. (n=5).

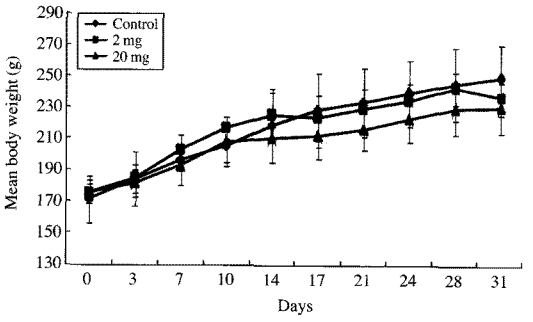

Figure 2. Changes in the body weights of female rats following oral administration of D/DA for 30 days. Results are the means ± S.D. (n=5).

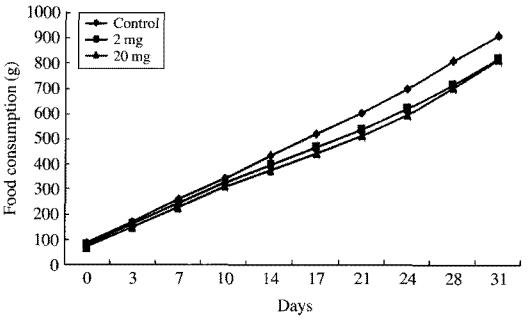

Figure 4. The cumulative food consumption of female rats administered D/DA orally for 30 days. Results are the means ± S.D. (n=5).

signs of toxicity or change in general behavior or other physiological activities (data not shown).

Subacute Toxicity Study

In the subacute toxicity test doses of 2 or 20 mg/kg body weight of D/DA, given orally for 30 days, did not death of the rats. No significant changes in the body weight were observed in the D/DA treated groups throughout the study period (Figures 1, 2). The food consumption of rats was higher than in the control rats. The 2 or 20 mg/kg body weight groups of D/DA increased food consumption for 30 days (Figures 3, 4). The effects of D/DA on the organ weights are summarized in Table 1. The organ weights were unaltered in the experimental groups when compared with the control group, which indicates that D/DA was not toxic in these vital organs. The results of the biochemical analyses of the serum of both male and female rats are shown in Table 2. The triglyceride (TG)[12] was found to be significantly increased in treated groups when compared to the control group. The hematological results (Table 3) were also assessed after 30 days of oral administration of D/DA. These results of the urinalysis are presented in Table 4. There were no significant changes observed in hematological parameters and urinalysis.

Discussion

Herbal medicines are comprised of plants or plant parts that are used for their scent, flavor or therapeutic properties. There has recently been increased interest in the use of herbal medicines. Herbal medicines are often sold as tablets, capsules, powders, teas, extracts and fresh or dried plants. Herbal medicines have also begun to be utilized in clinical therapy, which has resulted in increased demand for these remedies. However, some medicines can cause health problems, some are not effective and some may interact with other

Table 1. Absolute and relative organ weights in rats treated orally with D/DA for 30 days. Relative organ weights were calculated as the absolute organ weight /body weight × 100. All values represent the means ± S.D. (n=5); Differences among group were analyzed by a *t*-test; A.W., Absolute organ weight (g); R.W., Relative organ weight (%).

Sex		Male			Female		
Dose (mg/kg)		Control	2	20	Control	2	20
No. of rats		5	5	5	5	5	5
Heart	A.W.	1.21±0.07	1.14±0.122	1.33±0.044	0.84±0.089	0.85±0.043	0.86±0.046
	R.W.	0.52±0.031	0.48±0.044	0.55±0.046	0.49±0.050	0.47±0.026	0.49±0.017
Liver	A.W.	12.71±0.622	12.88±0.363	12.68±0.635	7.10±0.126	7.17±0.111	7.06±0.098
	R.W.	4.33±0.317	4.19±0.287	4.08±0.375	3.28±0.372	2.80±0.226	3.37±0.237
Spleen	A.W.	0.82±0.082	0.84±0.048	0.83±0.089	0.57±0.045	0.68±0.012	0.72±0.067
	R.W.	0.27±0.026	0.27±0.035	0.26±0.037	0.25±0.034	0.26±0.016	0.34±0.042
Stomach	A.W.	2.15±0.123	2.41±0.230	2.31±0.256	1.98±0.090	2.11±0.069	2.15±0.057
	R.W.	0.64±0.034	0.74±0.084	0.71±0.107	0.85±0.088	0.79±0.032	0.98±0.077
Lung	A.W.	1.42±0.208	1.47±0.211	1.47±0.134	1.20±0.064	1.20±0.107	1.14±0.089
	R.W.	0.52±0.073	0.50±0.078	0.49±0.071	0.58±0.038	0.48±0.045	0.55±0.045
Kidney-L	A.W.	1.32±0.073	1.31±0.114	1.29±0.062	0.81±0.053	1.01±0.101	0.96±0.025
	R.W.	0.54±0.025	0.51±0.040	0.49±0.041	0.44±0.052	0.46±0.074	0.53±0.030
Kidney-R	A.W.	1.29±0.090	1.33±0.068	1.33±0.043	0.79±0.030	0.99±0.092	0.95±0.025
	R.W.	0.51±0.030	0.48±0.031	0.47±0.030	0.40±0.046	0.41±0.060	0.49±0.024
Adrenal Gland-L	A.W.	0.03±0.010	0.02±0.010	0.03±0.011	0.03±0.008	0.03±0.013	0.04±0.013
	R.W.	0.01±0.003	0.01±0.002	0.01±0.003	0.01±0.004	0.01±0.005	0.02±0.006
Adrenal Gland-R	A.W.	0.02±0.010	0.03±0.010	0.03±0.008	0.04±0.009	0.03±0.015	0.03±0.005
	R.W.	0.01±0.002	0.01±0.003	0.01±0.002	0.01±0.004	0.01±0.006	0.01±0.003
Prostate	A.W.	1.24±1.118	1.14±0.051	1.41±0.312			
	R.W.	0.32±0.026	0.34±0.029	0.40±0.069			
Ovary	A.W.				42.04±0.747	41.88±0.687	41.78±0.672
	R.W.				17.64±1.320	15.46±0.607	18.42±0.991

Table 2. Serum biochemical analysis of rats administered D/DA orally for 30 days. *Significantly different from the control at $P<0.01$; Results are the mean ± S.D. (n=5); HDL, high density lipoprotein; T-CHO, total cholesterol; T-BIL, total bilirubin; TG, triglyceride, AST, aspartate aminotransferase; ALT, alanine aminotranferase.

Item	Sex	Male			Female		
	Dose (mg/kg)	Control	2	20	Control	2	20
HDL (mg/dL)		44.60±16.07	43.4±14.12	30.4±17.70	57.67±4.93	48.20±25.29	56.00±20.70
T-CHO (mg/dL)		75.00±13.34	68.60±12.34	75.00±15.68	97±14.32	91.80±14.79	96.80±23.30
T-BIL (mg/dL)		0.54±0.15	0.68±0.10	0.52±0.16	0.50±0.10	0.54±0.11	0.42±0.04
Creatinine (mg/dL)		0.28±0.08	0.32±0.04	0.34±0.09	0.37±0.06	0.32±0.04	0.32±0.04
TG (mg/dL)		70.00±15.89	85.20*±23.50	76.20±12.08	50.67±10.54	51.80±11.89	65.40*±9.94
AST (U/L)		75.20±19.79	78.60±7.30	76.40±4.39	69.67±0.58	67.00±14.42	69.60±15.21
ALT (U/L)		34.60±3.71	39.00±9.57	42.20±11.82	30.67±9.02	31.80±10.26	39.60±12.40

drugs. Therefore, experimental screening is important to ascertain the safety and efficacy of herbal products, as well as to establish the active component of this herbal remedies[13].

In this study, treatment of rats with D/DA at a dose of 200 or 2,000 mg/kg body weight did not induce any lethal or toxic symptoms. In addition, there was no mortality observed in response to treatment with the maximum dose level of 2,000 mg/kg body weight, which is the high dose recommended by the Organization for Economic Cooperation and Development (OECD). Therefore, further dosing to estimate the LD_{50} of the drug was not performed[14]. Taken together, these results suggest that the D/DA does not cause any apparent acute toxicity.

When the subacute toxicity was evaluated, administration of D/DA at doses of 2 and 20 mg/kg body weight every 24 h for 30 days did not result in the death of any animals. According to the OECD guideline, if an acute toxicity test at one dose level of at least 2,000 mg/kg body weight produces no observable toxic effects, a full study of the effects of a dose of 20

Table 3. Hematological analysis of rats administered D/DA orally for 30 days. Results are the mean±S.D. (n=5); WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet.

Item	Sex	Male			Female		
	Dose (mg/kg)	Control	2	20	Control	2	20
WBC ($\times 10^3/\mu L$)		7.64±0.29	7.36±0.31	7.41±0.31	6.45±0.26	6.57±0.45	6.74±0.28
Neutrophils (%)		38.39±2.29	35.85±2.35	33.94±2.00	31.83±1.51	31.57±1.53	30.55±1.30
Lympocytes (%)		56.58±2.19	57.78±0.88	58.26±1.47	63.40±0.53	63.76±2.73	63.66±1.95
Monocytes (%)		6.90±0.26	6.73±0.21	6.64±0.28	6.72±0.63	6.99±0.23	6.53±0.24
Eosinophils (%)		0.79±0.16	0.63±0.12	0.70±0.13	0.70±0.22	0.71±0.23	0.74±0.21
RBC ($\times 10^6/\mu L$)		7.94±0.39	7.81±0.41	7.89±0.33	7.54±0.53	7.45±0.27	7.44±0.43
Hgb (g/dL)		15.44±1.28	15.14±0.40	15.12±0.53	14.6±0.69	14.02±0.12	14.34±0.68
Hct (%)		39.80±3.17	39.20±1.03	39.78±1.68	38.5±1.56	38.72±1.94	38.10±1.84
MCV (fl)		50.08±1.90	50.04±1.45	51.1±1.11	51.06±1.60	51.66±0.21	50.54±1.82
MCH (pg)		19.40±0.78	19.44±0.75	19.18±0.51	19.37±0.57	19.54±0.40	19.34±0.36
MCHC (g/dL)		38.76±0.62	38.9±1.10	38.06±0.54	37.93±0.25	37.38±0.41	37.72±0.22
PLT ($\times 10^3/\mu L$)		1155.80±101.49	1074.40±85.32	1080.80±46.12	1192.00±119.53	1214.00±85.54	1144.00±94.09

mg/kg body weight given once daily for 14 days can be used to evaluate subacute toxicity[15]. However, a progressive increase in body weight of male and female rats was observed over the experimental period in both treatment groups, which may indicate improved nutritional status of the animal. The growth response observed in this study occurred as a result of increased food intake (Figures 3, 4), although there was no significant difference in the food intake of animals treated with 2 or 20 mg/kg when compared to the control group. Because no deaths were recorded in the acute toxicity study and no changes were observed in animal behavior or organ weight in response to any of the doses administered, it can be claimed that D/DA is non-toxic.

After treatment with D/DA for 30 days, there were no significant changes in the biochemical activities of the serum of male or female rats. An increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT)[16], and alkaline phosphatase (ALP) would have indicated hepatocyte damage[17]. The normal serum creatinine levels (Table 2) indicate that D/DA did not interfere with renal function[18], which implies that administration of D/DA at the doses evaluated in this study had no effect on the heart and liver tissue. However, the levels of TG in the plasma of the treated female rats increased in response to treatment with D/DA at doses of 2 and 20 mg/kg body weight. This may have occurred due to modification of the D/DA structure. D/DA can be easily modified into decursinol using NaOH, KOH, K_2CO_3 and $NaHCO_3$[19]. In addition, when TG reagents are added to the serum *in vitro*, D/DA can be modified into decursinol, after which it functions as glycerophosphate. Although the serum TG levels of the treated groups increased, these changes were not statistically significant.

There were no significant changes of hematological parameters in the levels of white blood cell (WBC)[20], red blood cell (RBC)[21], hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), neutrophils, lympocytes, monocytes, and eosinophils when compared to the control group. D/DA does not affect circulating red blood cells, hematopoiesis, or leucopoiesis[22]. The urinary parameters did not differ significantly between the treated groups and the control group, regardless of gender. Furthermore, the histopathology results revealed that all of the organs exhibited normal architecture, which indicates that D/DA was not toxic.

The present results suggest that, at the oral doses administered, D/DA is non-toxic. The present study can be regarded as a preliminary investigation; however, it is necessary to conduct further studies to establish the toxicity of D/DA. Prospective studies should include an evaluation of the reproductive toxicity, genetic toxicity, mutagenicity, and carcinogenicity of D/DA.

Materials & Methods

Drug and Chemicals

D/DA was extracted from *A. gigas* Nakai and determined to have a purity of 95% by HPLC. Hematological analyses were conducted using a Cell-dyn 3700 (Abbott, USA). Biochemical analyses were conducted using a Fuji dri-chem 3500i (Fujifilm, Japan). Kits for the determination of urinalysis were purchased from Yeongdong Electronic Coporation (Uriscan, Korea). All other chemicals and solvents used were of analyti-

Table 4. Urinalysis in rats treated orally with D/DA for 30 days.

Item	Sex	Male			Female		
	Dose (mg/kg)	Control	2	20	Control	2	20
	No. of rats	3	3	3	3	3	3
Bilirubin	Normal (−) 0.5 mg/dL (+) 1 mg/dL (++) 3 mg/dL (+++)	3	3	3	3	3	3
Blood	Normal (−) 10 RBC/μL (+) 50 RBC/μL (++) 250 RBC/μL (+++)	3	3	3	3	3	3
Nitrate	Negative (−) Positive (+)	3	3	3	3	3	3
Specific gravity	1.000 (−) 1.005 (±) 1.010 (+) 1.020 (++) 1.025 (+++) 1.030 (++++)	 2 1	3	3	3	3	3
Leukocytes	Normal (−) 25 WBC/μL (+) 75 WBC/μL (+) 500 WBC/μL (+)	 3	1 2	3	2 1	3	3
Bilirubin	Normal (−) 0.5 mg/dL (+) 1 mg/dL (++) 3 mg/dL (+++)	3	3	3	3	3	3
Blood	Normal (−) 10 RBC/μL (+) 50 RBC/μL (++) 250 RBC/μL (+++)	3	3	3	3	3	3
Nitrate	Negative (−) Positive (+)	3	3	3	3	3	3
Specific gravity	1.000 (−) 1.005 (±) 1.010 (+) 1.020 (++) 1.025 (+++) 1.030 (++++)	 2 1	3	3	3	3	3
Leukocytes	Normal (−) 25 WBC/μL (+) 75 WBC/μL (+) 500 WBC/μL (+)	 3	1 2	3	2 1	3	3

cal grade.

Animals

Male and female SD rats (240 ± 10 g) were used in this experiment. Prior to each experiment, the animals were fasted overnight while provided with free access to water. Animals were kept in a temperature-controlled environment (23 ± 2°C) with a 12 h light-dark cycle and provided with food and water *ad libitum*.

Acute Toxicity Study

Bioassays were conducted according to the World Health Organization guideline for evaluation of the safety and efficacy of herbal medicines[23]. The animals were divided into a control group and two treatment groups, each of which consisted of ten animals (5 males and 5 females). The control group received the sterilized water while the treated group received either 200 or 2,000 mg/kg body weight of D/DA by gavage.

These doses were chosen because they were 10 and 100 times higher than the effective normal doses. 2 mg/kg body weight of D/DA would be effective to improve the streptozotocin-induced diabetic rats and the gout of rats in former experiments (data not shown). The animals were closely observed for the first 24 hr for any toxic symptoms and then for 2 weeks for any signs of behavioral changes or mortality.

Subacute Toxicity Study

The animals were divided into a control group and two treatment groups, each of which consisted of ten animals (5 males and 5 females). The control group received the sterilized water and each treated group received D/DA at a dose of either 2 or 20 mg/kg body weight by gavage once daily for 30 days. During the treatment period, the animals were weighed and the food intake was monitored every 3 days.

Clinical Test Parameters

At 30 days, the animals were fasted for 12 hr, after which they were anesthetized with ether and blood was collected from the jugular vein into two tubes. The blood in one tube, which contained EDTA, was immediately analyzed for hematological parameters including WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT and the presence of neutrophils, lympocytes, monocytes, and eosinophils. The blood in the other tube, which did not contain any additives, was centrifuged at 4,000 rpm and 4°C for 10 min, after which it was analyzed for biochemical parameters such as the levels of plasma ALT, AST, creatinine[24], TG, total bilirubin (T-BIL), total cholesterol (T-CHO)[25], and high density lipoprotein cholesterol (HDL). In addition, selected organs (liver, heart, spleen, left kidney, left lung, intestine, and muscle) were removed for macroscopic analysis. Furthermore, the heart, liver, spleen, stomach, lung, left kidney, right kidney, left adrenal gland, right adrenal gland, prostate, and ovaries were removed and evaluated for changes in weight. All organs were washed and transferred to an ice-cold saline solution before histopathological examinations and weight measurement.

Urine Analysis

During the last week of exposure, urinalysis of 3 males and 3 females per group was conducted using fresh urine to determine the pH, the levels of protein[26], glucose, ketone bodies, bilirubin, urobilinogen, nitrite, leukocyte, and the specific gravity contents.

Statistical Analysis

Data are presented as the means ± standard deviation (S.D.). Results were analyzed statistically using a student's *t*-test. A $p < 0.01$ was considered to indicate statistical significance.

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